SUMMARY OF DATA FOR CHEMICAL SELECTION

Plumbagin
481-42-5

BASIS OF NOMINATION TO THE CSWG

Plumbagin is brought to the attention of the CSWG as a potentially toxic natural product. For many years, humans have been exposed to folk remedies containing plumbagin as the active ingredient. Recent changes in dietary supplement regulations now permit products containing plumbagin to be marketed as long as no therapeutic claims are made.

There is much evidence to suggest that plumbagin may have potential as a chemotherapeutic or chemopreventive agent. Plumbagin has also been evaluated by the Developmental Therapeutics Program, National Cancer Institute (NCI) in its screening panel against HIV-1. However, plumbagin is a redox cycling compound that generates superoxide, a reactive species that can damage various biomolecules. Very limited information on the toxicity of plumbagin, including evidence that it may be an abortifacient, raises questions as to whether plumbagin will have too low a margin of safety to exploit its anticarcinogenic properties.

SELECTION STATUS

ACTION BY CSWG: 6/22/99

Studies requested:

Preliminary studies:

- Mechanistic studies predictive of carcinogenic/anticarcinogenic potential
- Metabolism studies
- Mouse lymphoma assay
- Mammalian mutagenicity assay

Follow-up:

Based on the results from the preliminary studies, select either juglone or plumbagin for carcinogenicity testing

Priority: High

Rationale/Remarks:

- Natural yellow pigment found in plants of the *Plumbagineae* and *Droseracea* families.

- Redox-cycling agent that also induces DNA cleavage; demonstrated reproductive toxicity.
- Given the close relationship between juglone and plumbagin, chronic studies of only the more reactive compound are recommended.

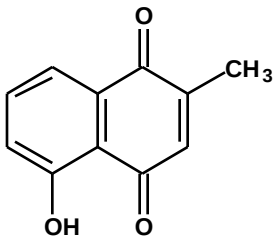
- Existing information on the carcinogenic/anticarcinogenic potential of the two compounds is insufficient to determine which compound is more reactive.

- NCI will conduct the mouse lymphoma assay.

CHEMICAL IDENTIFICATION

CAS Registry Number:	481-42-5
Chemical Abstracts Service Name:	1,4-Naphthalenedione, 5-hydroxy-2-methyl- (9CI)
Synonyms and Trade Names:	5-Hydroxy-2-methyl-1,4-naphthoquinone; 2-methyl-juglone; plumbagin; plumbagone
Structural Class:	Bicyclic; naphthoquinone

Structure, Molecular Formula and Molecular Weight:



$C_{11}H_8O_3$ Mol. wt.: 188.18

Chemical and Physical Properties:

Description:	Yellow needles (Merck, 1997)
Melting Point:	78-79%C (Merck, 1997)
Solubility:	Slightly soluble in hot water; soluble in alcohol, acetone, chloroform, benzene, and acetic acid (Merck, 1997)
Reactivity:	Highly toxic, corrosive (Sigma-Aldrich, 1999)

Technical Products and Impurities: Plumbagin is available at a purity of 95+% from TCI America (1998) and 99+% from Acros Organics (1997).

EXPOSURE INFORMATION

Production and Producers: Plumbagin is a natural product found in many plants. A yellow naphthoquinone pigment, it occurs in plant roots as a colorless combined form that can be processed to plumbagin by acid treatment (Botanical Dermatology Database, 1999a).

Plumbagin is characteristic of plants in the tribe *Plumbagineae*. According to the Botanical Dermatology Database (1999a), the following species have been reported to yield plumbagin:

- *Ceratostigma*
- *Limonium*
- *Limonium carolinianum (sea lavender)*
- *Plumbagella*
- *Plumbago* (leadwort)
- *Plumbago capensis* (cape leadwort)
- *Plumbago coerulea*
- *Plumbago europaea*
- *Plumbago rosea*
- *Plumbago pulchella*
- *Plumbago scandens*
- *Plumbago zeylanica* (Ceylon leadwort)
- *Statice*
- *Statice limonium* (English sea lavender).

Plumbagin is also contained in members of the *Droseraceae* (Sundew) family. This family of insectivorous plants comprises some 105 species in four genera. *Drosera* L., the largest genus, is usually found in acid bogs. The following *Drosera* species have been reported to contain plumbagin:

- *D. anglica* Hudson
- *D. auriculata* Backh.
- *D. binata* Labill.
- *D. capensis* L.
- *D. cistiflora* L.
- *D. indica* L.
- *D. intermedia* Hayne
- *D. longifolia* L.

- *D. peltata* Smith
- *D. rotundifolia* (round-leaved sundew)
- D. whitakeri Planchon
- D. ramentacea Burchell.

Other genera in the *Droseraceae* family are *Aldrovanda vesiculosa* L., a free floating aquatic plant, *Dionaea muscipula* Ellis, also known as Venus' Fly Trap, and *Drosophyllum lusitanicum* Link, commonly called Portugese sundew (Botanical Dermatology Database, 1999b; Hoffman, 1999).

Eight US producers or distributors of plumbagin are listed by Chem Sources (Chemical Sources International, 1999). Five sources of plumbagin were identified from chemical catalogs; these included Acros Organics, Aldrich, Indofine Chemical Company, Inc., Sigma, and TCI America (Acros Organics, 1997; TCI America, 1998; Indofine Chemical Company, 1999; Sigma-Aldrich, 1999).

Plumbagin is not listed in EPA's TSCA Inventory.

Use Pattern: Quinones, including plumbagin, have an important function in nature. The stability of the semiquinone radical permits quinones to conduct electrons from two-electron donors to single-electron acceptors in the respiratory pathways of both prokaryotic and eukaryotic cells (Imlay & Fridovich, 1992).

In the presence of quinones, molecular oxygen can act as a univalent electron acceptor, generating superoxide, a reactive species that can damage various biomolecules. Some organisms take advantage of this property by excreting plumbagin into their immediate environment, where the plumbagin enters and poisons competitors (Imlay & Fridovich, 1992).

Pure plumbagin is used primarily in research designed to exploit its properties as a superoxide generator, an antibiotic, and an antineoplastic agent. Between 1976 and 1999, 32 patents involving plumbagin were obtained in the United States. Many of these patents involve polymer scale prevention agents (US Patent and Trademark Office, 1999).

Impure extracts of plumbagin from *Plumbagineae* and *Droseraceae* have long traditions of use in folk medicines. *Plumbago zeylamica* L., *Plumbago rosea* L., and *Plumbago europaea* L. have been used in China and other Asian countries for the treatment of cancer, rheumatoid arthritis, dysmenorrhea, and contusion of extremities. The round-leaved sundew has been used in North America to remove warts, corns, keratoses, and freckles. The crushed leaves of *Drosera burnammii* Vahl are considered to be a powerful rubefacient in Hindu medicine. *Plumbago europaea*, or leadwort, has long been used in France to relieve toothache (Itoigawa *et al.*, 1991; Grieve, 1995; Botanical Dermatology Database, 1999b).

The antibacterial properties of plumbagin are well documented in traditional medicine. The stem barks of *Pera benesis*, containing plumbagin as the most active compound, are employed by the Chimane Indians in the Bolivian Amazon as treatment of cutaneous leishmaniasis. Sundew reportedly has great benefit in treating bronchitis and whooping cough (Itoigawa *et al.*, 1991; Fournet *et al.*, 1992; Hoffman, 1999).

Plumbago zeylanica is grown as a perennial herb in the plains of Bengal and southern India. Extracts of the root have been reported to be a powerful poison which, when given internally or applied to the ostium uteri, causes abortion (Premakumari *et al.*, 1977; Barghava, 1984).

The Venus' flytrap extract, Carnivora, produced in Germany, is perhaps the most controversial use of a product containing plumbagin. The Food and Drug Administration (FDA) considers Carnivora to be an unapproved new drug and has issued an import alert requesting the automatic detention of imported Carnivora N-DMP, injectable, and Carnivora N-DMP, drops. The FDA has noted that the manufacturer, Carnivora Forschungs GmbH, "double" invoices these items, misdeclaring them as vitamins, Nectar or juice, while another invoice inside the shipping boxes identifies them as drugs for the treatment of cancer, chronic diseases, and HIV infection (FDA, 1992).

Venus Fly Trap Herbal Extract is available in the United States from Vital Health Products in Muskego, Wisconsin. Vital Health Products stresses that this extract is sold only as a food supplement (Venus Fly Trap - Carnivora, 1999).

Human Exposure: No reports of occupational exposure to plumbagin during its production or processing were found in the available literature. No listing was found for plumbagin in the National Occupational Exposure Survey (NOES), which was conducted by the National Institute for Occupational Safety and Health (NIOSH) between 1981 and 1983.

Human exposure to plumbagin occurs primarily through the consumption of the extracts described above under the section on use.

Environmental Occurrence: As indicated earlier, plumbagin is present in members of the *Plumbagineae* and *Droseraceae* families of plants. *Plumbagineae* are found in Africa, many parts of Asia, and in Europe. *Droseraceae* are found in many temperate and tropical regions of the world, notably in Australia, New Zealand, and South Africa (Botanical Dermatology Database, 1999a,b).

Regulatory Status: No standards or guidelines have been set by NIOSH or OSHA for occupational exposure to or workplace allowable levels of plumbagin. Plumbagin was not on the American Conference of Governmental Industrial Hygienists (ACGIH) list of compounds for which recommendations for a threshold limit value (TLV) or biological exposure index (BEI) are made.

EVIDENCE FOR POSSIBLE CARCINOGENIC ACTIVITY

Human Data: No epidemiological studies or case reports investigating the association of exposure to plumbagin and cancer risk in humans were identified in the available literature.

Animal Data: No 2-year carcinogenicity studies of plumbagin were identified in the available literature. Acute toxicity values are shown in Table 1.

Table 1. Acute toxicity data for plumbagin

Route	Species	LD_{50} (mg/kg)	Comments	Reference
Oral	Rat	65	72 hr observation period	Premakumari *et al.*, 1977
Oral	Mouse	40	72 hr observation period	Premakumari *et al.*, 1977
Oral	Mouse	8.51	14 day observation period	Singh & Udupa, 1997
Oral	Mouse	16	72 hr observation period	Krishnaswamy & Purushothaman, 1980
Intraperitoneal (ip)	Mouse	15		Merck, 1997
ip	Mouse	16	72 hr observation period	Krishnaswamy & Purushothaman, 1980

According to Singh and Udupa (1997), plumbagin has many toxic side effects including diarrhea, skin rashes, increases in white blood cell and neutrophil counts, increases in serum phosphatase and acid phosphatase levels, and hepatic toxicity.

Short-Term Tests: *Genotoxicity.* Plumbagin has been tested for mutagenicity, chromosomal aberrations, and DNA damage in several standard assays. Table 2 presents data on the Ames *Salmonella* reverse mutation assay conducted with or without metabolic activation by the S-9 microsomal enzyme fraction.

Table 2. Mutagenic activity of plumbagin

S. typhimurium strain	Results without S-9	Results with S-9	Sensitivity of strain	References
TA97	negative; positive	positive	sensitive to frameshift mutations	Durga *et al.*, 1992; Hakura *et al.*, 1994
TA98	negative(n=3)	negative; positive	sensitive to frameshift mutations	Matsushima *et al.*, 1986 Durga *et al.*, 1992; Hakura *et al.*, 1994; Edenharder & Tang, 1997
TA100	negative (n=2)	negative (n=2); positive	sensitive to base-pair substitution	Matsushima *et al.*, 1986 Durga *et al.*, 1992; Hakura *et al.*, 1994
TA102	negative (n=2)	negative	sensitive to base-pair substitution	Durga *et al.*, 1992; Hakura *et al.*, 1994; Watanabe *et al.*, 1998
TA104	positive	positive	sensitive to oxidative mutagens	Hakura *et al.*, 1994
TA1535	negative	negative	sensitive to base-pair substitution	Hakura *et al.*, 1994
TA1537	negative	positive	sensitive to frameshift mutations	Hakura *et al.*, 1994
TA1538	negative	negative	sensitive to frameshift mutations	Hakura *et al.*, 1994
TA2637	positive (n=2)	positive (n=3)	detects bulky DNA adducts; sensitive to frameshift mutations	Tikkanen et al., 1983; Matsushima *et al.*, 1986; Hakura *et al.*, 1994
TA2638	negative		genetic properties similar to TA102 but fewer spontaneous revertent colonies	Watanabe *et al.*, 1998

n = number of independent investigations

The mutagenic activity of plumbagin in *Escherichia coli* has also been examined. Watanabe and coworkers (1998) reported that plumbagin was negative in *E. coli* strains WP2/pKM101 and WP2 uvrA/pKM101 using the plate incorporation method without metabolic activation. Farr and coworkers (1985) assayed with *E. coli* AQ634 cells, measuring Trp- ø Trp+ reversion frequency, and reported that plumbagin was not mutagenic in stationary-phase cells but was moderately mutagenic in exponential-phase cells.

The somatic mutation and recombination *w/w+* eye assay in *Drosophila melanogaster* was used for genotoxic evaluation of several reactive oxygen species inducers; plumbagin was evaluated as positive (Gaivão *et al.*, 1999).

Plumbagin acted like a mitotic inhibitor in onion root tips by arresting cell division as expressed by the occurrence of mitotic anomalies such as polypoidy, micronucleus, anaphase bridges, giant cells, and stickiness and lagging of chromosomes (Krishnaswamy & Purushothaman, 1980).

Santhakumari and coworkers (1980) examined the effects of plumbagin on chick embryo fibroblast cultures. The most predominent effects seen were an arrest of cell growth and proliferation and a decrease in mitotic index with accumulation of cells in metaphase. These changes, evident at concentrations as low as 0.1 g, were associated with chromosomal aberrations. At higher concentrations, nuclear and cytoplasmic vacuoli-zation, disintegration of cytoplasm, karyopyknosis, and nuclear polymorphism occurred. The authors concluded that low plumbagin at lower concentrations behaves like a spindle poison by inhibiting entry of cells into mitosis, but at higher concentrations, it also exhibits radiomimetic nucleotoxic and cytotoxic effects.

Metabolism: No information on the metabolism, distribution, or excretion of plumbagin was identified in the available literature.

Other Biological Effects: *Anticarcinogenic Activity*. Male F344 rats administered plumbagin at 200 ppm in the diet for two weeks beginning one week before azoxymethane (AOM) injection had a lower incidence (41%) ($P<0.05$) and multiplicity (0.48 Å 0.62) ($P<0.01$) of tumors in the small intestine than those administered AOM alone (68% and 1.04 Å 0.62). These data suggested to the authors that plumbagin could be a promising chemopreventive agent for human intestinal neoplasia (Sugie *et al.*, 1998).

Plumbagin administered orally to male Wistar rats at 4 mg/kg bw induced regression of 3-methyl-4-dimethyl aminoazobenzene (3-Me-DAB) induced hepatoma. The effects of treatment with plumbagin on the rate of glycolysis and gluconeogenesis in tumor-bearing rats were also measured. Hexokinase, phosphoglucoisomerase, and aldolase levels increased in hepatoma-bearing rats, but they decreased to near-normal levels in animals administered plumbagin. Levels of the gluconeogenic enzymes, glucose-6-phosphatase and fructose-1,6-diphosphatase decreased ($P<0.001$) in hepatoma-bearing animals but increased in the animals treated with plumbagin (Parimala & Sachdanandam, 1993).

Plumbagin was screened for chemotherapeutic activity and activity against HIV-1 by the NCI's Developmental Therapeutics Program (Krishnaswamy & Purushothaman, 1980; NCI, 1999). Plumbagin has been tested as a chemotherapeutic agent vs several tumor cell lines in *in vitro* assays and in tumor xenografts. The results are summarized in Table 3.

Plumbagin induced mammalian topoisomerase II-mediated DNA cleavage *in vitro* possibly through the formation of a cleavable complex. The DNA cleavage induced by plumbagin was different from the cleavage patterns induced with other known topoisomerase II-active drugs. A DNA-unwinding assay with T4 DNA ligase showed that plumbagin intercalates into DNA (Fujii *et al.*, 1992).

Table 3. Tests of plumbagin as a chemotherapeutic agent

Tumor cell line	Test Conditions	Route of Plumbagin Administration	Results	Reference
Mouse leukemia L5178Y	*in vitro*, 5 days duration	N.A.	IC50 = 6.6 x 10^{-7} M	Suzuki *et al.*, 1998
Ehrlich ascites tumors	ip injection in BALB/c mice	oral	dose-dependent increase in lifespan	Singh & Udupa, 1997
P_{388} lymphocytic leukemia	ip injection in mice	no data available	active	Krishnaswamy & Purushothaman, 1980 (work performed at NCI)
L1210 lymphoid leukemia	ip injection in mice	no data available	not active	Krishnaswamy & Purushothaman, 1980 (work performed at NCI)
Dalton's ascitic lymphoma	ip injection in Swiss mice	ip	inhibition of tumor cell growth and enhanced mean survival time	Kavimani *et al.*, 1996
B16F1 melanoma	intradermal implants in C57 mice	ip	plumbagin and controlled release formulations of plumbagin were ineffective	Kini *et al.*, 1997
Ehrlich ascites tumors and sarcoma-180	ascites tumors - ip injection; solid tumors - intradermal implants; in BALB/c mice	intravenous (iv) injection	free plumbagin and controlled release plumbagin significantly increased tumor volume doubling time	Naresh *et al.*, 1996
MC-induced fibrosarcomas	injection in axilla of Wistar rats	ip and oral	ED_{50} = 0.75 mg/kg (ip & oral)	Krishnaswamy & Purushothaman, 1980

N.A. = not applicable; IC50 = molar concentration required to inhibit tumor cell growth 50 percent; ED_{50} = effective dose required to inhibit tumor cell growth 50 percent; MC = methylcholanthrene.

Antimutagenicity. Five naphthoquinones (chimaphilin, 5,8-hydroxy-1,4-naphthoquinone, juglone, plumbagin, menadione) were tested for their antimutagenic potencies with respect to mutagenicities induced by 2-nitrofluorene (2NF), 3-nitrofluoranthene (3-NFA) and 1-nitropyrene (1-NP) in *S. typhimurium* TA98. All five were potent antimutagens

irrespective of the presence of methyl or hydroxyl functions. Plumbagin, however, showed exceptional antimutagenicity (Edenharder & Tang, 1997).

Adaption to stress. Toxicity to bacteria, including *E. coli, S. typhimurium, and Bacillus subtilis*, can be used to measure stress adaption to redox-cycling agents, such as plumbagin. In stress adaption, the bacteria responds to pretreatment with the redox-cycling agent by changing its pattern of gene expression, dramatically increasing the synthesis of specific proteins. The induction of these proteins protects the cell against additional exposure to the stressor at a higher concentration. *E. coli* AB1157 cells pretreated with plumbagin showed enhanced survival upon exposure to high concentrations of plumbagin (Farr *et al.*, 1985; Hartford & Dowds, 1991; Prieto-Alamo *et al.*, 1993).

Cross protection by plumbagin against lethal levels of a different stressor has also been observed (Hartford & Dowds, 1991). When *E. coli* strain BW9109, which lacks active oxygen defense systems, was pretreated with a sublethal dose of plumbagin, it showed resistance to a lethal dose of selenious acid; resistance was not observed after pretreatment with hydrogen peroxide (H_2O_2) (Hiratsu *et al.*, 1991). Non-lethal concentrations of plumbagin also protected *B. subtilis* JH642 cells against the lethal effects of H_2O_2 (Hartford & Dowds, 1991).

Mechanistic studies in bacteria. Because of its properties as a redox cycling compound, plumbagin has been used as a model compound in bacterial assays developed to measure damage from reactive oxygen species (ROS).

All aerobically growing micro-organisms encounter ROS, including the superoxide anion ($O_2^{\cdot-}$), H_2O_2, and the hydroxyl radical, which are capable of damaging cellular constituents. Bacteria posess a sophisticated network of inducible defenses against ROS. In addition to predamage protective mechanisms, most notably superoxide dismutases (SODs) and catalase,

postdamage repair mechanisms have been identified. DNA enzymes that are important in repairing oxidative damage include exonuclease III, endonuclease IV, and several DNA glycosylases. Several tests that manipulate these antioxidant defenses to potentiate genotoxicity have been developed (Jamieson *et al.*, 1994; Farr *et al.*, 1985; Prieto-Alamo *et al.*, 1993; Kato *et al.*, 1994).

E. coli mutants provide excellent model for the study of oxidative damage. Mutants defective in the *katE* locus lack the catalase designated as hydroperoxidase II (HPII). The expression of HPII also requires a functional *katF* gene which is a regulator of both exonuclease III and the HPII enzyme. Hydroperoxidase I, HPI, is a bifunctional enzyme exhibiting both catalase and peroxidase activities. The gene encoding HPI is *katG*. *E. coli* also contains two forms of SOD, manganese SOD (Mn-SOD) and iron SOD (Fe-SOD) (Prieto-Alamo *et al.*, 1993*).* Plumbagin is a potent inducer of Mn-SOD in *E. coli* (Farr *et al.*, 1985).

The Ara test quantifies the role of peroxide in mutagenesis by measuring the frequency of mutation to L-arabinose resistance in mutant *E. coli*. *E.coli* strains deficient in catalase were constructed by mutation in *kat* genes. *E. coli* strains with diminished levels of SOD were also constructed. The mutant strains were compared to their SOD or catalase-proficient parent counterpart with respect to the genotoxic effects of plumbagin. The mutagenicity of plumbagin was much higher in SOD-deficient than in catalase-deficient bacteria (Prieto-Alamo *et al.*, 1993).

Although the Ara tests using plumbagin suggest a role for SOD, other studies present evidence that the damage and the repair response induced by plumbagin is distinct from the damage and repair brought about by exposure to H_2O_2 or to agents that induce the SOS response. These differences were demonstrated in several ways:

- *E. coli* AB1157 cells pretreated with SOS inducers withstood challenge doses of far uv irradiation far better than untreated cells; for those pretreated with plumbagin, no effect was observed.

· Plumbagin did not induce prophage induction in a lambda lysogen and did not increase β-galactosidase activity in a *dinD::Mu d(lac* Ap) fusion strain, known SOS responses.

· *polA* and *recA* mutants, hypersensitive to killing by H_2O_2, were only slightly more sensitive to killing by plumbagin than was the isogenic wild type, while *xthA* was even more refractive.

· Cells damaged by plumbagin could reactivate riboflavin/light damaged phage more efficiently than could untreated cells, a response not seen for H_2O_2 damaged cells.

These results led the authors to conclude that the toxicity of plumbagin is, at least in part, a function of DNA damage. Plumbagin, unlike H_2O_2, did not cause single-strand breaks to any large extent. While the nature of the DNA damage was not identified, the authors noted that it differed from than that caused by H_2O_2 and that the cellular responses to plumbagin and H_2O_2 were distinct (Farr *et al.*, 1985).

Ono and coworkers *(*1991) examined the sensitivity and adaptive responses of the *E. coli* mutants UM196, RPC501, and TN1005, which lack systems for active oxygen defense, to plumbagin. The mutant strain UM196 showed a higher sensitivity to plumbagin than AB1157, its wild strain, but TN1005 was not more sensitive than the wild strain, CSH7. When UM196 was pretreated with a sublethal dose of plumbagin, it did not acquire an adaptive response such as was seen for pretreatment with H_2O_2. These results also suggested the possibility that active oxygen species generated by plumbagin may damage DNA besides a pathway *via* H_2O_2.

Derivatives of *E. coli* WP2s (*uvrA trpE*) defective for glycosylase activity and/or an adaptive response to superoxide-induced oxidative stress were constructed to compare the mutability to various reactive oxygen-generating compounds. Induction of Trp+ reversion was assayed both in the presence and absence of plasmid pKM101; plumbagin was not mutagenic to any strain. No induction of the SOS response was detected by treatment with plumbagin (Kato *et al.*, 1994).

Orser and coworkers (1995) designed an *in vitro* assay to give an indication of mechanisms of toxicity quickly and easily. The system consists of the *lacZ* structural gene fused to and under the control of a wide variety of *E. coli* and *S. typhimurium* stress gene promoters integrated into the *E. coli* chromosome. Plumbagin was tested against sixteen bacterial stress promoter-*lacZ* fusion strains and consistently induced genes associated with hydroperoxides and superoxide radical generating agents. Plumbagin also induced a gene associated with DNA damage and changes in DNA superstructure.

In contrast to the studies in *E. coli* , Jamieson and coworkers (1994) conducted tests in *Saccharomyces cerevisiae* cells suggesting that the toxic action of plumbagin is due to the production of superoxide anions. Using disruption mutations in the genes encoding the two superoxide dismutases, Cu/ZnSOD (*SOD*1) and mitochondrial MnSOD (*SOD*2), these authors showed that the *sod*1 mutant was 100-fold more sensitive to plumbagin than its isogenic parent. In contrast, the sensitivity of the *sod*2 strain to plumbagin was indistinguisable from that of the wild-type strain,S150-2B. The sensitivities of the *sod*1 and *sod*2 strains to H_2O_2 were unchanged. Thus, Cu/ZnSOD was the prinicipal superoxide dismutating activity under the growth conditions tested.

Effects on Microsomal Enzymes. Plumbagin exhibited a potent, dose-dependent inhibitory activity against aromatase cytochrome P450 in human placental microsomes. However, plumbagin showed relatively weak reducing effects in the presence of microsomal membranes, suggesting to the authors that the inhibitory effects on monooxygenase reactions were not due to the formation of superoxide radicals (Muto *et al.*, 1987).

Reproductive Effects. Plumbagin has demonstrated reproductive toxicity in male and female animals. Teratogenic effects were not seen in limited studies.

Only one of 12 female Long-Evans rats intubated with plumbagin at 10 mg/kg for 10 days conceived, bearing a litter of five pups. All 12 control animals conceived, producing an average litter size of six pups. One animal in the plumbagin group died of hemorrhage that the authors suspected was caused

by competitive inhibition of vitamin K activity, needed for the synthesis of clotting factors (Azad Chowdhury *et al.*, 1982).

On the twentieth day of gestation, pregnant albino rats were inspected for the number of implantation sites. Four of six animals receiving plumbagin-containing albumin microspheres and two of six receiving plumbagin or plumbagin entrapped on a lipid layer failed to conceive. All six control animals conceived. The number of implantation sites in individual rats was also higher in controls than in the plumbagin-exposed animals. No difference in the histological structures of the uteri of control and treated animals was seen. The ovaries of the treated group showed clear inhibition of growth of graffian follicules and degeneration of the mature follicles and corpus luteum. According to the authors, the antifertility action of plumbagin seemed to be related to its antiovulatory action (Kini *et al.*, 1997).

Plumbagin administered by intubation to albino female rats at 10 mg/kg for 15 days significantly inhibited mating and prolonged duration of estrus cycle and diestrus phase. Plumbagin showed a dose-related abortifacient activity in rats administered 5-20 mg/kg orally from day 5 to day 11 of pregnancy. At 10-20 mg/kg from days 1 to 5 of pregnancy, plumbagin caused a significant anti-implantation effect. No gross teratogenic effects were noticed in pups born to female rats that had received 5 or 10 mg/kg plumbagin from days 1 to 5 of pregnancy (Premakumari *et al.*, 1977).

Plumbagin given orally at 10 mg/kg for 10 days to adult female rats of the Holtzman strain caused a highly significant decease in the weight of ovaries as compared with the controls (Santhakumari & Suganthan, 1980).

Plumbagin administered ip at a dose of 10 mg/kg for 60 days caused selective testicular lesions in dogs. The wet weights of testes and epididymides were decreased. In addition, the seminiferous tubule and Leydig cell nuclei diameter were significantly decreased and cellular heights of epididymides were drastically curtailed (Bhargava, 1984).

Oral administration of plumbagin to male gerbils at 10 mg/day for 20 days caused a decrease in the wet weight of seminal vesicle and prostate glands. The cell height of the secretory epithelium was also decreased, and little secretion in the lumen of these glands was observed (Bhargava, 1984).

Plumbagin caused a decrease in the number of spermatids, resting and pachytene spermatocytes, and a significant reduction in seminiferous tubule and Leydig cell nuclei diameter when given orally to immature Wistar rats at 10 mg/kg for 32 days (Bhargava, 1986).

Cardiotonic Action. Plumbagin produced a triphasic inotropic response in guinea-pig papillary muscle. Plumbagin did not cause any positive inotropy under anoxic conditions, and the positive inotropic effect was markedly inhibited by oxidative phosphorylation uncouplers (Itoigawa *et al.*, 1991).

Hypolipidemic and Antiatherosclerotic Effects. When administered to hyperlipidaemic rabbits, plumbagin reduced serum cholesterol and LDL-cholesterol by 53 to 86 percent and 61 to 91 percent, respectively. Further, plumbagin treatment prevented the accumulation of cholesterol and triglycerides in liver and aorta and regressed atheromatous plaques of the thoracic and abdominal aortas (Sharma *et al.*, 1991*).*

Structure-Activity Analysis: Several types of naphthoquinones are found in nature, all having the same nucleus but different substituents and substitution patterns. Pharmacologically interesting activities of naphthoquinones, such as antimicrobial, antifungal, and antitumor activities have been reported.

The absence of a lipophilic isoprenoid side chain confers significant aqueous solubility upon toxic quinones, such as plumbagin, and ensures their access to both cytosolic and membrane-bound electron carriers. By intercepting electrons and then transferring them to molecular oxygen, these quinones act as intracellular sources of superoxide and hydrogen peroxide. Enzymes, DNA, and membranes can be damaged by these oxidants, resulting in growth inhibition or cell death (Imlay & Fridovich, 1992).

Unsubstituted naphthoquinones generally show no mutagenicity in the *Salmonella* mutation assay in the presence or absence of S-9 metabolic activation systems. However, derivatives containing one or more hydroxyl groups and/or methoxyl groups have been shown to be mutagenic in *S. typhimurium* in the presence of S-9. Because humans are exposed to these mutagens, the relations between the chemical structures and mutagenicities of the naphthoquinones have been studied (Matsushima *et al.*, 1986).

For this project, three structurally related naphthoquinones (1,4-naphthoquinone, lawsone, and menadione) were screened for relevant information on mutagenicity and carcinogenicity. Information on additional compounds (dichlone, lapachol, naphthazalin, and shikonin) that was available from the literature search and retrieval conducted for plumbagin is also presented. Finally, in Table 4, the structure of juglone, another chemical under consideration, is presented.

No 2-year carcinogenicity studies of plumbagin, 1,4-naphthoquinone, lawsone, or menadione were identified in the available literature. RTECS describes 1,4-naphthoquinone as an equivocal tumorigenic agent (lungs, thorax and respiration, skin and appendages) on the basis of a 29 week skin painting study in mice (NLM, 1999a). RTECS describes menadione as an equivocal tumorigenic agent on the basis of tumors at the application site in two skin painting studies in mice, one at 1860 mg/kg/27 weeks and the other at 8400 mg/kg/21 weeks (NLM, 1999a).

Table 4. Structurally related naphthoquinones

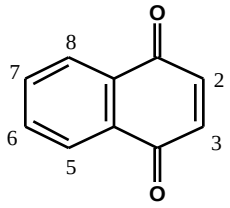
Structure	Naphthoquinone	Substituent
	Plumbagin	2-CH_3 , 5-OH
	Juglone	5-OH
	1,4-Naphthoquinone	None
	Menadione	2-CH_3
	Lawsone	2-OH
	Dichlone	2,3-Cl
	Lapachol	2-CH_3, 3-$CH_2CH=C(CH_3)_2$
	Naphthazalin	5,8-OH
	Shikonin	2-$CHOHCH_2CH=C(CH_3)_2$

Table 5 provides a summary of information found on the genotoxicity of plumbagin, 1,4-naphthoquinone, lawsone, and menadione.

Table 5. Information on genotoxicity of selected naphthoquiones

Chemical Name	Mutagenicity Data
Plumbagin [481-42-5]	*S. typhimurium* without S-9 metabolic activation*:* positive in strains TA104 & TA2637; negative in strains TA98, TA100, TA102, TA1535, TA1537, TA153 & TA2638; inconsistent in TA97 *S. typhimurium* with S-9 metabolic activation: positive in strains TA97, TA104 TA1537 & 2637; negative in TA102, TA1535 & TA1538; inconsistent in TA9 & TA100 (Tikkanen *et al.*, 1983; Matsushima *et al.*, 1986; Durga *et al*., 1992; Hakura *et al.*, 1994; Edenharder & Tang, 1997; Watanabe *et al*., 1998) *E. coli*: negative without S-9 in strains WP2/pKM101 & WP2uvrA/pKM101; positive in strain AQ634 (exponential phase cells) (Farr *et al.*, 1985; Watanabe *et al*., 1998) *Drosophila*: positive in somatic and recombination test (Gaivão *et al*., 1999) Onion root tips: positive for polypoidy, micronuclei, anaphase bridges, giant cells, sticky chromosomes (Krishnaswamy & Purushothaman, 1980) Chick embryo fibroblasts: positive for decrease in mitotic index, chromosomal aberrations, nucleo- & cytotoxicity (Santhakumari *et al.*, 1980)
1,4-Naphthoquinone [130-1-4]	*S. typhimurium* with arochlor induced S-9: negative in strains TA97, TA98 & TA100 *S. typhimurium* with phenobarbital (PB)/5,6-benzoflavone (BF) induced S-9: positive in TA97, TA100, TA102, TA104 & TA2637; negative in TA98, TA1537 & TA1538 *S. typhimurium* without S-9: positive in TA104 and TA2637; negative in TA97

	TA98, TA102, TA1535, TA1537 & TA1538; inconclusive in TA100 (NLM, 1999b) Human lymphocytes: induced micronuclei (NLM, 1999a)

Lawsone [83-72-7]	*S. typhimurium* with arochlor induced S-9: positive in strain TA1537; negative in TA98, TA100 & TA1535 *S. typhimurium* with PB/BF induced S-9: negative in TA98, TA100, TA102, TA104, TA1535, TA1537, TA1538 & TA2637 *S. typhimurium* without S-9: negative in TA98, TA100, TA102, TA104, TA1535, TA1537, TA1538 & TA2637 (NLM, 1999b) *In vivo* mouse (ip): induced micronuclei (NLM, 1999a) *Drosophila*: negative for sex-linked recessive lethal/reciprocal translocation (NTP, 1999)
Menadione [58-27-5]	*S. typhimurium* with arochlor induced S-9: negative in strains TA98, TA100, TA1535, TA1537 *S. typhimurium* with PB/BF induced S-9: positive in TA98 & TA2637; negativ in TA97, TA100, TA102, TA104, TA1535, TA1537 & TA1538 *S. typhimurium*: without S-9: positive in TA 97 & TA2637; negative in TA 97A, TA98, TA102, TA1535, TA1537 & TA1538; inconclusive in TA100 & TA104 (NLM, 1999b) *Drosophila*: positive, specific locus (NLM, 1999a); marginally positive in somatic and recombination test (Gaivão *et al.*, 1999) Human fibroblast: DNA damage (NLM, 1999a) Hamster cells: DNA damage (NLM, 1999a) Rat liver: DNA damage (NLM, 1999a) Mouse embryo: morphological transformation (NLM, 1999a) Hamster lung: positive cytogenetic analysis (NLM, 1999a) Human lymphocytes: induced sister chromatid exchanges (SCE) (NLM, 1999a Hamster lung: induced SCE (NLM, 1999a)

Hakura and coworkers (1994) conducted extensive tests of mutagenicity on additional naphthoquinones. This information is presented in Table 6 for dichlone, naphthazalin, and lapachol.

Table 6. Mutagenicity of some naphthoquinones for 9 *S. typhimurium* strains with or without S-9 mix

Compound	S-9	TA1535	TA100	TA1538	TA98	TA1537	TA2637	TA97	TA102	TA104
Dichlone	wo	-	pos	-	-	-	-	-	pos	pos
Dichlone	w	-	pos	-	-	-	pos	pos	pos	pos
Naphthaz-alin	wo	-	pos	-	-	-	pos	pos	pos	pos
Naphthaz-alin	w	-	pos	-	-	pos	pos	pos	pos	pos
lapachol	wo	-	-	-	-	-	-	-	-	-
lapachol	w	-	-	-	-	-	-	-	-	-

pos = positive; - = negative; w = with S-9; wo = without S-9

Other information related to the potential toxicity of the naphthoquinones listed in Table 4 includes:

- Plumbagin and juglone inhibited aromatase cytochrome P450; shikonin and naphthazarin were weak inhibitors; lawsone was negative (Muto *et al.*, 1987).

- Juglone and 1,4-naphthoquinone exhibited potent activities reducing cytochrome c; naphthazalin, shikonin, and plumbagin showed only weak effects (Muto *et al.*, 1987).

- Hydroxyl derivatives of naphthoquinone were mutagenic to *S. typhimurium* TA2637 and TA98 but not to TA100; a methyl group at position 2 of junglone enhanced mutagenicity, while a methyl group at position 7 decreased mutagenicity (Matsushima *et al.*, 1986).

- Plumbagin and shikonin, but not lawsone or lapacol, induced topoisomerase II mediated DNA cleavage (Fujii *et al.*, 1992)

- Shikonin, lawsone, and lapacol did not intercalate DNA while plumbagin did (Fujii *et al.*, 1992).

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